

Importance of Respiratory Viruses in Acute Otitis Media

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INTRODUCTION

Otitis media is a major worldwide health care problem of childhood. In addition to the distress that it brings on the patient and the family, otitis media also causes an enormous economic burden to the society in terms of physician visits, medications, surgical procedures, and absences from work, school, or day care (40, 79, 97). Acute otitis media (AOM) is the most frequent reason for outpatient antibiotic therapy in children in the United States and most other developed countries (54, 90). According to a recent estimate, AOM accounts for 33% of all visits to physicians and approximately 40% of all antibiotic use in children younger than 5 years (135). The peak incidence of AOM occurs in children between 6 and 24 months of age (4, 107, 122). By the age of 2 years, 70% of children have experienced at least one episode of AOM, and approximately 20% of children have suffered four or more attacks of AOM (4, 122).

AOM is generally considered a bacterial infection that is treated with antibiotics. However, bacterial pathogens cannot be isolated from the middle-ear fluid (MEF) in approximately 30% of AOM cases (19, 31). Although the causes of such sterile cultures might include technical inadequacies resulting

in loss of bacterial growth or the presence of fastidious bacterial organisms, the substantial proportion of AOM cases without a proven bacterial etiology has initiated an intensive search for the role of viruses in the etiopathogenesis of this condition. During the past 2 decades, research in this area has produced strong evidence for the crucial role of viruses in the development of AOM.

EPIDEMIOLOGICAL STUDIES

Association of Respiratory Viruses with AOM

The occurrence of AOM shows an extensive seasonal variation. In temperate regions, the incidence rates are highest in the wintertime and lowest during the summer months, which parallels the incidence of upper respiratory infections (107, 108). The occurrence of AOM peaks in the youngest children, who suffer an average of six to eight respiratory infections per year, and it decreases rapidly after the age of 2 to 3 years, at which time also respiratory infections become less prevalent (107, 122). During the first years of life, boys appear to have more respiratory infections than girls, and the incidence of AOM is also higher in boys (3, 91).

Everyday clinical experience clearly indicates that AOM is closely associated with upper respiratory tract infections. In a study of 363 children with newly diagnosed AOM, symptoms of upper respiratory infection were present in 94% of the patients

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Healthy child**Increased spread of infection**

Type of day care
Number of siblings
Season
Type of housing
Area of residence

Decreased defense against infection

Young age
Male gender
Passive smoking
Short breast-feeding
Immune disorders

Respiratory virus infection**Anatomical, physiological, and immunological factors**

Cleft palate
Nasopharyngeal dimensions
Down syndrome
Patulous Eustachian tube
Adenoid tissue
Young age
Passive smoking
Race or ethnicity
History of AOM in siblings
Short breast-feeding
Immune disorders
Nasal allergy

Acute otitis media

FIG. 1. Proposed sites of influence of some risk factors for AOM.

at the time of diagnosis (5). Several signs and symptoms that are traditionally associated with AOM, e.g., fever, purulent rhinitis, cough, poor appetite, vomiting, diarrhea, and tiredness, have been shown to be unspecific to AOM but to be caused by the concurrent viral infection (67, 82, 96, 131).

Extensive clinical studies have documented the tight association between AOM and laboratory-documented viral respiratory infections. In a 14-year follow-up study among children attending day care, viral infections, especially those caused by respiratory syncytial virus (RSV), influenza virus, and adenovirus, conferred a greater risk of AOM than did colonization of the nasopharynx with *Streptococcus pneumoniae* or *Haemophilus influenzae* (72). Another study carried out in a pediatric department included 4,524 cases of AOM in children seen during a 6-year period (108). There was a significant correlation between AOM and laboratory-documented epidemics of respiratory viruses, especially RSV epidemics.

Several factors such as young age, day care attendance, male gender, number of siblings, passive smoking, and short duration of breast-feeding have been identified as important risk factors for AOM (3, 47, 101, 122, 130). However, it is evident from several studies that, in fact, all of the above risk factors for AOM predispose individuals to upper respiratory infection which, in turn, can be considered the most important factor

predisposing to AOM (Fig. 1). In most cases, AOM can be clearly regarded as a complication of a preceding or concomitant upper respiratory infection. This concept was corroborated by a recent study of 596 infants monitored from birth to 6 months of age (43). Respiratory tract infection was found as the most important predictor for AOM early in life, unquestionably outweighing all other risk factors, including day care attendance.

The rate of development of AOM during upper respiratory infection is largely dependent on the age of the child and potentially also on the type of virus causing the infection. The comparative ability of different viruses to predispose an individual to AOM has not been convincingly determined because of important confounding factors, especially age, in clinical studies. The frequency of AOM in children with respiratory infections has usually ranged between 10 and 50%, with the highest rates associated with RSV infection (72, 108, 129). As a rough estimate across all viral infections, AOM can be considered to occur in approximately 20% of children with upper respiratory infection (71, 121).

Temporal Development of AOM during Viral Respiratory Infection

Although in many children AOM is diagnosed concurrently with upper respiratory infection, the development of AOM only occurs after a certain time interval after the onset of the viral infection. Knowledge about this time interval is important, because it represents the window of opportunity for intervention to prevent the development of AOM in children in whom the symptoms of the viral infection are already present. Two studies including a total of more than 1,200 children have specifically attempted to determine the temporal development of AOM during the course of the upper respiratory infection (Fig. 2). In the first of these, 204 episodes of AOM in children less than 4 years old attending day care were analyzed (66). The highest incidence of AOM was observed on day 3 after the onset of upper respiratory infection, and the median day for diagnosing AOM was day 4. Of all AOM episodes, 54% were diagnosed during the first 4 days and 75% were diagnosed during the first week after the onset of respiratory symptoms. No significant differences were observed in the time to development of AOM between subgroups of children grouped according to age, gender, history of otitis media, smoking in the household, previous adenoidectomy, or presence of tympanostomy tubes.

The second study included 250 episodes of AOM in children with a mean age of 3 years (81). The incidence of AOM peaked on day 4, and 63% of cases were diagnosed during the first week after the onset of symptoms. Age, gender, or history of AOM recurrences did not affect the temporal development of AOM. In children with two separate episodes of AOM during the study, the time to development of AOM during the first occurrence did not correlate with the time lag during the second episode. These findings suggest that the type of virus may have a greater impact on the temporal development of AOM than any host-related individual factor.

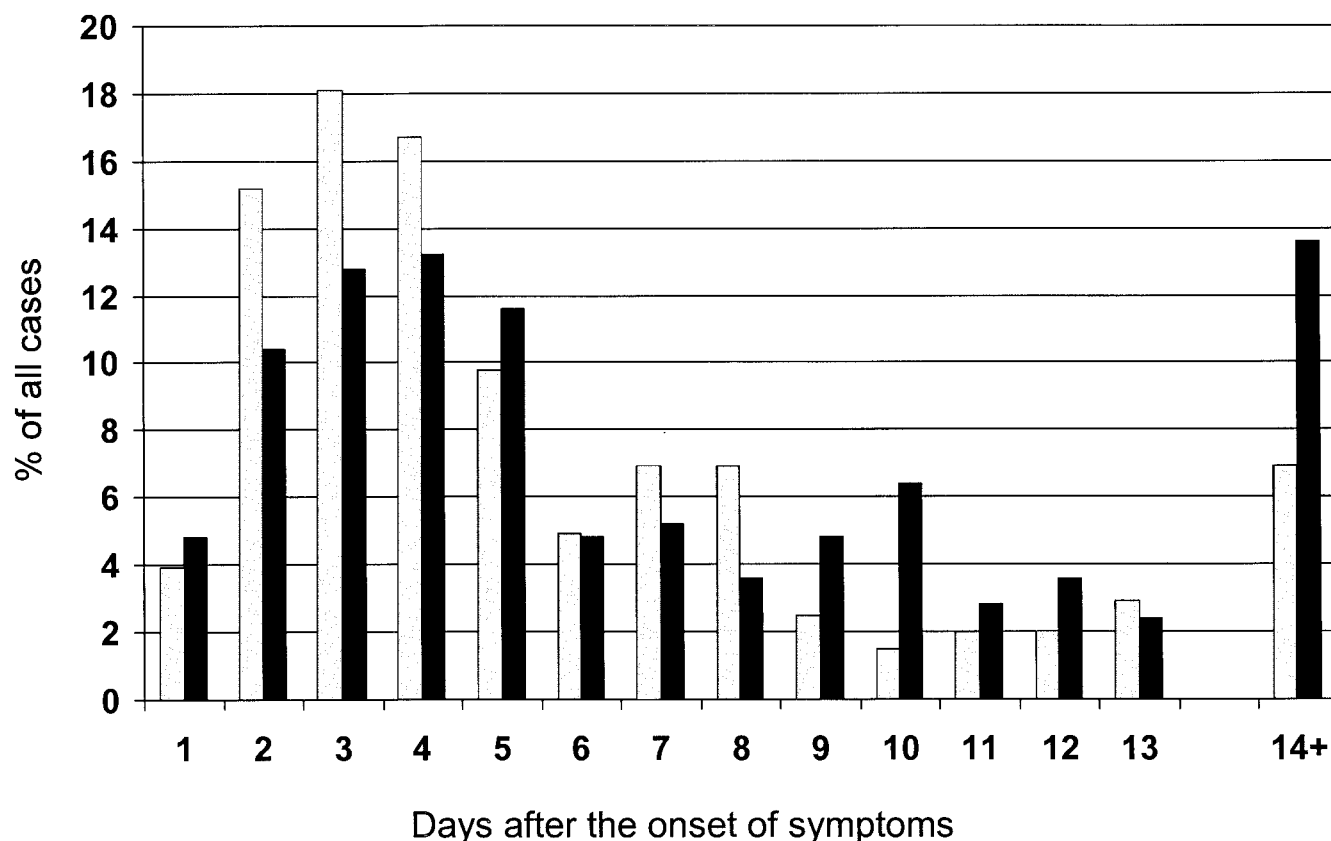


FIG. 2. Temporal development of AOM after the onset of upper respiratory symptoms. Data derived from reference 66 (grey bars) and reference 81 (black bars).

Presence of Viruses in Nasopharyngeal Specimens from Children with AOM

Considering the clear connection between AOM and laboratory-documented viral upper respiratory infections, it could be expected that viruses had been found in the nasopharynxes of virtually all children with AOM. However, the rates of detection of respiratory viruses have conventionally ranged between 30 and 50% (5, 6, 7, 35, 37, 80, 113). The most frequently found viruses have been RSV; influenza A and B viruses; parainfluenza type 1, 2, and 3 viruses; and adenovirus. The relatively low rates of viral detection in nasopharyngeal specimens from children with AOM have raised doubts about the extent of viral involvement in the development of this disease. With the increasing availability of PCR-based assays, however, it has become obvious that the low rates have been caused by underdetection of existing viruses in studies where viral detection has been based only on viral culture and/or antigen detection methods. PCR has proved especially valuable in diagnosing rhinovirus infections, for which other methods have been suboptimal (8, 74, 87). Rhinoviruses are estimated to account for 30 to 40% of all respiratory illnesses also in children (91), and a recent follow-up study demonstrated that by the age of 2 years more than 90% of children have experienced a laboratory-confirmed rhinovirus infection (17). Because of the existence of more than 100 different serotypes of rhinoviruses, antigen detection techniques cannot be rou-

tinely used to detect these viruses. In a study utilizing PCR for the detection of rhinovirus, RSV, and coronavirus only, at least one of these viruses was identified in nasopharyngeal aspirates from 62% of children with AOM (106). Another recent study searched for viruses using antigen detection for RSV; influenza A and B viruses; parainfluenza type 1, 2, and 3 viruses; and adenovirus—together with PCR for rhinovirus, enterovirus, and coronavirus. Viral infection could be documented in the nasopharyngeal specimens of 90% of the children with AOM (T. Heikkinen, A. Ruohola, M. Waris, and O. Ruuskanen, Abstr. 4th Extraordinary Int. Symp. Recent Adv. Otitis Media, abstr. 133, 2001).

ROLE OF VIRUSES IN THE PATHOGENESIS OF AOM

Eustachian Tube Dysfunction

Viral infection of the upper respiratory tract results in congestion of the nasal and nasopharyngeal mucosa. Congestion in and around the nasopharyngeal orifice of the Eustachian tube leads to dysfunction of the tube, which is considered the most important factor in the development of AOM (18). Eustachian tube dysfunction results in (i) impairment of pressure equilibration between the nasopharynx and the middle ear cavity; (ii) decreased drainage into the nasopharynx of secretions produced in the middle ear; and (iii) loss of protection of the middle ear from nasopharyngeal secretions. Even at the nor-

mal stage, the muscular opening function of the Eustachian tube is poorer in children than in adults; this function improves with increasing age (27, 28).

Animal studies. Experimental studies in animals have provided important information on the role of viruses in the pathogenesis of AOM. Compared with studies in humans, the clear advantage of animal experiments is the possibility to have a better control over various confounding factors. Most experimental studies of viral involvement in otitis media have been performed with chinchillas. Chinchillas are well suited for otitis media research because their middle ears are easily accessible; middle ear infections can be produced by intranasal inoculation of microbes; the disease remains localized to the middle ear; and, unlike many other animal species, chinchillas are not susceptible to naturally occurring middle ear infections (57). In one of the early studies, chinchillas were inoculated intranasally with influenza A virus, *S. pneumoniae*, or both of these pathogens (58). Otitis media developed in 4% of the animals inoculated with influenza virus alone and in 21% of those inoculated with pneumococci alone, whereas it developed in 67% of chinchillas inoculated with both influenza virus and *S. pneumoniae*. The synergistic effect between viruses and bacteria has also been demonstrated in infant chinchillas inoculated with adenovirus and nontypeable *H. influenzae* (120). All animals inoculated with both of these pathogens developed more-severe otitis media than those receiving either pathogen alone. The most severe and prolonged episodes of otitis media were observed in chinchillas that were initially inoculated with adenovirus, followed by inoculation of *H. influenzae* 7 days after the virus. It seems, however, that the synergistic effect of viruses and bacteria is not a constant feature across all types of microbes, because a subsequent experiment in chinchillas using *Moraxella catarrhalis* instead of *H. influenzae* did not demonstrate a similar synergy (9).

Intranasal inoculation of chinchillas with influenza A virus was shown to result in negative middle-ear pressure that was not observed in animals inoculated with *S. pneumoniae* only (2). The extent of epithelial damage in the Eustachian tube caused by influenza A virus infection was later demonstrated in a histopathologic study in which the development of negative middle-ear pressure was associated with marked ciliary damage, disappearance of ciliated epithelial cells, and increased accumulation of mucus and cellular debris in the tubal lumen (59). The substantial damage to ciliated epithelial cells in the Eustachian tube was also shown after direct inoculation of influenza A virus into the middle ear of chinchillas, in which the restoration of the mucosal architecture was not observed until 4 weeks after viral inoculation (38). Impairment of the Eustachian tube function following experimental infections with influenza A virus or adenovirus has also been demonstrated in many other animal species, including guinea pigs and ferrets (10, 22, 99).

Human studies. Clinical studies of both children and adults have provided strong evidence for a causal role of respiratory viruses in the disruption of normal Eustachian tube function. Several studies have documented the impairment of Eustachian tube function in children with upper respiratory infections (20, 26). In a prospective tympanometric study of preschool children, significant negative middle-ear pressures were measured in 13% of asymptomatic children, but the rate of

abnormal tympanograms increased to 75% during upper respiratory infections (112). More than 80% of the negative middle-ear pressures were already detectable by the second day of illness, and 97% were detectable after 4 days of illness. These findings are well in accordance with the clinical studies demonstrating that the peak incidence of AOM occurs 3 to 4 days after the onset of respiratory symptoms (66, 81). A recent study among children 2 to 12 years old corroborated the earlier findings of the effect of viral infection on Eustachian tube function (138). In children with normal tympanograms before the onset of illness, abnormal negative middle-ear pressures occurred at least once during the 2 weeks after the onset of symptoms in 66% of cases. Approximately 90% of the tympanometric abnormalities were observed during the first week of illness. In another tympanometric follow-up study of children through an entire winter season, high negative middle-ear pressures during upper respiratory infections preceded the development of middle-ear effusion (92).

The development of AOM as a complication of upper respiratory infection is uncommon in adults. Nonetheless, experimental viral challenge studies carried out among adult volunteers have provided much detailed information about the effect of viral infection on Eustachian tube function. Substantial deterioration of normal Eustachian tube function has developed in 50 to 80% of adults following intranasal challenge with rhinoviruses, and significant negative middle-ear pressures have been observed in 30 to 50% of the subjects (24, 46, 89). Even higher rates of abnormalities have been demonstrated in adults infected with influenza A virus. By the fourth or fifth day after intranasal challenge with influenza A virus, Eustachian tube dysfunction has occurred in more than 80% of volunteers, and significant middle-ear underpressures occurred in 60 to 80% of the volunteers (23, 44). In one of the influenza challenge studies, one volunteer developed AOM as a complication, and PCR analysis of the MEF revealed both influenza A virus and *S. pneumoniae* (23). A recent experimental study of adults challenged with RSV showed that by day 6 after viral inoculation, more than half of the subjects had abnormal negative middle-ear pressures (25). The frequent development of substantial negative middle-ear pressures has also been observed during naturally occurring rhinovirus infections in adults (49).

The detailed mechanisms by which viral infection causes Eustachian tube dysfunction are still incompletely understood. However, extensive research during the recent years into the role of inflammatory mediators in the pathogenesis of viral upper respiratory infections has disclosed some potential mechanisms. Numerous studies, both in vivo and in vitro, have demonstrated that respiratory viruses are fully capable of inducing the release of several inflammatory mediators in the nasopharynx. Increased concentrations of mediators such as kinins, histamine, leukotrienes, interleukins (interleukin-1 [IL-1], IL-6, and IL-8), tumor necrosis factor, and RANTES have been found in nasal secretions of patients with respiratory infections (94, 98, 111, 133, 136), and the concentrations in nasal secretions of many of these substances are correlated with the severity of the symptoms (53, 128, 141). Experimental studies in adult volunteers have, in turn, documented that after intranasal challenge, several of these mediators induce Eustachian tube dysfunction (45). The inflammatory mechanisms

triggered by viral infection are, however, extremely complex and interrelated, affecting each other in a time-dependent manner (12, 104). It is likely that despite rapidly accumulating data on the host inflammatory response during a viral infection, several important factors contributing to the inflammatory process in the nasopharyngeal mucosa still remain to be determined.

Alteration of Host's Immune Defense

Some viruses, particularly influenza A virus, are known to induce polymorphonuclear leukocyte dysfunction, which could be thought to increase the risk of secondary bacterial infections (1). Several studies have shown that influenza A viruses decrease the oxidative, chemotactic, secretory, and bactericidal activities of neutrophils (reviewed in reference 1). In experimental studies with chinchillas, this neutrophil dysfunction was observed 4 to 8 days after influenza virus inoculation, but it did not occur after inoculation of *S. pneumoniae* alone (2). The highest incidence of pneumococcal otitis media occurred when the bacteria were inoculated 4 days after the virus, i.e., just before the emergence of virus-induced neutrophil dysfunction. Furthermore, no difference was observed in the rate of otitis media between ears with and without a tympanostomy tube, suggesting that in this animal model neutrophil dysfunction contributed more to the pathogenesis of otitis media than did negative middle-ear pressure. Suppression of polymorphonuclear leukocyte function has also been shown in humans infected with influenza viruses (83, 115). Scarce clinical data are available on other effects of respiratory viral infections on the host's immune defense, but some studies have documented several changes in systemic immune and inflammatory parameters during infections with rhinovirus, RSV, or influenza virus (56, 73, 109, 118). For example, experimental rhinovirus infection of adult volunteers resulted in increased mitogen-stimulated production of IL-2 and gamma interferon by peripheral blood mononuclear cells, as well as increased natural killer cell-mediated cytotoxicity of the mononuclear cells on day 5 after rhinovirus challenge (73).

Bacterial Colonization and Adherence

In addition to eliciting various local and systemic immune and inflammatory responses in the host, viral infection of the upper respiratory tract appears to have a substantial impact on the nasopharyngeal bacterial flora. Pneumococcal colonization of the nasopharynx has been reported to be increased in adult patients infected with influenza A virus (134). Influenza A virus-induced promotion of nasopharyngeal colonization by *S. pneumoniae* has also been observed in chinchillas (127). However, chinchillas infected with adenovirus did not show enhanced colonization by pneumococci (127). In cotton rats, colonization with *H. influenzae* increased within 4 days of RSV infection (102). Additional, albeit indirect, evidence for the effect of viruses on the bacterial colonization of the nasopharynx was derived from a study of children with AOM, in whom the occurrence and the quantity of pathogenic bacteria in the nasopharynx were significantly increased during AOM episodes (50).

Viruses have also been reported to increase the adherence

of bacteria to epithelial cells. In laboratory experiments, influenza A virus infection significantly increased the binding of *S. pneumoniae* to HEp-2 cells compared with uninfected cells (48). Certain types of adenoviruses enhance the binding of pneumococci to human A549 cells (63). Further, RSV infection has been shown to increase the attachment of nontypeable *H. influenzae* to human respiratory epithelial cells (75). Increased adherence of *S. pneumoniae* and *H. influenzae* to pharyngeal cells has also been demonstrated in vivo in adult volunteers infected with influenza A virus (51). However, despite a considerable amount of new information on the interaction between viruses and bacteria on the cellular level, the detailed mechanisms by which bacteria colonizing the nasopharyngeal mucosa turn into disease-causing and even invasive organisms are still unknown. Based on available data, it seems likely that by various mechanisms the viral infection activates the epithelial cells, which could then result in increased adherence of bacteria to the cells (42). Recent discoveries on the molecular mechanisms have, however, clearly demonstrated that also other factors such as phase variation in the colonial opacity of pneumococci play an important role in epithelial adhesion and invasion (42, 126).

VIRUSES IN THE MIDDLE EAR

Presence of Viruses in MEF during AOM

The detection of viruses directly in the MEF of children with AOM is a central piece of evidence for the role of viruses in the pathogenesis of this disease. The first attempts to search for viruses in the MEF were carried out already in the 1950s and 1960s, when detection of viruses was limited to viral culture only (Table 1). Although viruses could be isolated from the MEF of several patients during influenza or RSV outbreaks (15, 16, 140), the general rate of viral detection remained lower than 5%, and in many studies viruses could not be found at all (61, 62, 64, 84, 125). Improvements in viral culture techniques and the development of various antigen detection methods during the subsequent decades substantially increased the rates of viral detection in the MEF. Since the 1980s, viruses have been detected in the MEF of children with AOM in approximately 20% of cases using viral culture and/or antigen detection techniques (6, 7, 33, 34, 35, 37, 80, 113, 114) (Fig. 3). In about one-third of virus-positive cases, the virus has been the sole pathogen found in the MEF. The most common viruses detected in the MEF in these studies were RSV, influenza viruses, adenovirus, and parainfluenza viruses, i.e., the same viruses that had been identified in the nasopharyngeal specimens from children with AOM. However, several other viruses, such as cytomegalovirus and herpes simplex virus, have also been isolated in the MEF specimens from children with AOM (36).

As with nasopharyngeal specimens, the advent of PCR technique has also dramatically increased the detection rates of viruses in MEF specimens. In one of the first PCR-based studies, the use of PCR for the detection of RSV yielded positive results in 53% of children with AOM, and the rate exceeded 80% in those children in whom RSV could be cultured in the nasopharyngeal secretions at the same time (100). In another study, the use of PCR for detection of rhinovirus,

TABLE 1. Detection of viruses in MEF by different viral diagnostic methods

Method	Refer- ence	Yr	No. of children or MEF specimens	No. (%) virus positive
Culture alone	140	1955	10	4 (40)
	61	1964	326	0 (0)
	16	1966	27	9 (33)
	84	1966	16	0 (0)
	125	1967	90	2 (2)
	15	1967	33	7 (21)
	64	1968	74	1 (1)
	62	1968	11	6 (55)
Total			587	29 (5)
Culture and/or antigen detection	80	1982	53	13 (25)
	114	1983	84	7 (8)
	113	1985	137	24 (18)
	37	1986	84	17 (20)
	7	1988	143	16 (11)
	6	1990	88	17 (19)
	34	1990	58	11 (19)
	35	1992	271	66 (24)
	33	1996	106	19 (18)
Total			1,024	190 (19)

RSV, and coronavirus revealed at least one of these viruses in the MEF specimens from 48% of children with AOM (106). The power of PCR in comparison with conventional methods was well demonstrated in a recent study of 65 MEF specimens from children with AOM (30). The investigators used multiplex PCR for the detection of RSV; influenza A and B viruses; and parainfluenza type 1, 2, and 3 viruses. Viruses had been previously found by culture in 29% of the MEF specimens, but the use of PCR increased the rate to 72%. Two-thirds of specimens that were negative by virus culture turned out to be positive by PCR. Such high rates of viral detection by PCR inevitably raise the question of the real clinical significance of these viruses in the middle ear. Theoretically, the extreme

sensitivity of the PCR technique could result in positive viral findings in the MEF even in cases where minute amounts of viral genomic materials have entered the middle ear along with nasal secretions.

Recent data from a study of the relative prevalences of viruses in the MEF of 456 children with AOM have provided additional evidence for the active role of at least some viruses in the pathogenesis of AOM (69). In children with AOM during laboratory-confirmed RSV infection, the same virus could be found in the MEF in 74% of the cases, a rate which was significantly higher than the corresponding ones for parainfluenza viruses (52%) or influenza viruses (42%). The relative prevalences of all these viruses, in turn, were higher than those for enteroviruses (11%) or adenoviruses (4%). These differences imply that while it is possible that some viruses get to the middle ear passively along with nasal secretions, some other viruses seem to actively invade the middle ear cavity. If the presence of all viruses in the MEF were the result of passive influx only, they should be detected in the MEF at roughly similar rates.

In the above study (69), the relative prevalence of rhinoviruses in the MEF could not be analyzed because PCR techniques were not used and the rates of rhinovirus detection by culture were too low for meaningful analysis. However, the impact of rhinoviruses may be equal to that of RSV, because a PCR-based study of children with AOM detected rhinoviruses in the MEF of 69% of children infected with this virus (106).

In approximately two-thirds of cases where virus has been detected in the MEF by culture or antigen detection, bacteria have also been identified, which indicates a mixed viral-bacterial infection. Overall, combined viral-bacterial infections have accounted for approximately 15% of all AOM cases. It is very likely, however, that this proportion does not represent the true occurrence of mixed infections, because it is obvious that underdetection of viruses is still very common. Apart from well-known problems in culturing some respiratory viruses, antigen detection and PCR also have their disadvantages in this context. The use of antigen detection or PCR requires specific antibodies or primers for each virus being searched for.

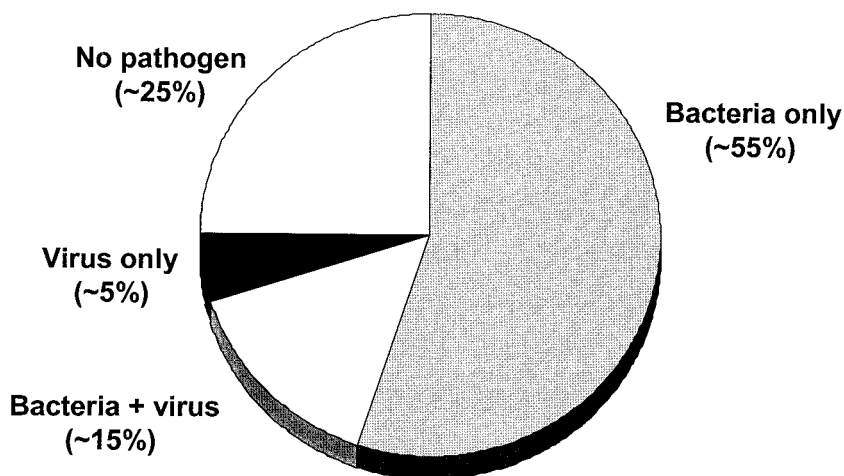


FIG. 3. Presence of bacteria and viruses in MEF specimens of children with AOM. Bacteria were detected by culture; viruses were detected by viral culture and/or antigen detection. Data derived from references 29, 34, 37, and 69.

Even with the availability of an extensive array of assays for different viral types, the relatively small volume of MEF would be a limiting factor for the detection of viruses. On the other hand, it has also been reported that the routine bacteriologic techniques are not sensitive enough for complete detection of bacteria in the MEF specimens (132).

Although clear synergism between certain viruses and bacteria has been shown in the development of AOM (58, 120), scarce data are available on potential relationships between types of viruses and bacteria in the MEF. One study of children with AOM has demonstrated a close connection between influenza virus and *S. pneumoniae* (69). In the MEF specimens that contained both bacteria and virus, *S. pneumoniae* was cultured significantly more often in MEF specimens containing influenza viruses than in those containing RSV or parainfluenza viruses.

Effect of Viruses on the Outcome of AOM

One of the major issues suggesting an active role for viruses in otitis media is that despite the use of wide-spectrum antibiotics that are effective against the causative bacteria, the clinical response to antibiotic treatment is often poor. This usually results in the change of the antibiotic and even the use of several courses of different antibiotics with the assumption that the poor response was due to bacterial resistance to the antibiotic used. Studies of children with persistent symptoms of AOM have shown, however, that resistant bacteria only account for approximately 20% of these cases (105, 123). It is logical to think that if the middle ear mucosa is infected with both bacteria and viruses simultaneously, eradication of the bacteria with antibiotics may not be sufficient to stop the inflammatory cascades in the middle ear, and the continuing viral infection may lead to more prolonged disease.

The results of clinical studies of viral AOM during the past decade suggest that viruses play an important role in the resolution of otitis media. In a study of 58 children with AOM, MEF samples were obtained by tympanocentesis both before the start of the antibiotic treatment and 2 to 4 days into the treatment (34). The bacteria found in the MEF were susceptible to the antibiotic used. At the time of the second tympanocentesis, bacteriologic failure was observed in 33% of the children who had both bacteria and virus in the initial MEF specimen. In children with only bacteria in the initial MEF specimen, the rate of bacteriologic failure was 3%; the difference was statistically significant. Similar results were obtained in a subsequent study of 271 children, in which otitis media persisted in a higher proportion of children with evidence of mixed viral-bacterial infection than in those with only bacteria in the MEF (35). Further, another study of 88 children with AOM involved 22 children who had persistent symptoms of AOM despite 2 days of antibiotic therapy (6). Bacteria were isolated in the MEF of only four of the children with poor response to antibiotic treatment, and only one of these isolates was resistant to the drug used. By using viral culture and antigen detection, viruses were found in the MEF of 32% of the children with clinical failure, compared with 15% of children in a comparison group with newly diagnosed untreated AOM. The follow-up of the children demonstrated that in children with prolonged symptoms, viruses were found in the

MEF in 44% of cases, whereas the corresponding rate was 11% in those with good response to the treatment.

Based on the recent findings about the varying activity of different viruses in invading the middle ear cavity (69), it could be hypothesized that the impact of different viruses on the clinical outcome of otitis media could also vary. Whether this is true is largely unknown, but one study has suggested that rhinoviruses may be more commonly associated with bacteriologic failure in the middle ear than are other respiratory viruses (119).

Mechanisms of Virus-Bacterium Interaction

Despite increasing evidence for interaction between viruses and bacteria, the detailed mechanisms by which the presence of viruses in the middle ear might interfere with the resolution of otitis media are still unclear. Some hypothetical virus-induced mechanisms that have been investigated include (i) production or inhibition of inflammatory mediators in the middle ear, (ii) delayed clearance of bacteria from the middle ear, and (iii) decreased penetration of antibiotics into the middle ear.

Inflammatory mediators. Clinical signs and symptoms of AOM are most likely caused by the host's inflammatory response to infection in the middle ear (110). Both bacteria and viruses are known to induce the production of cytokines and other inflammatory mediators at the sites of infection (94, 98, 133, 136, 141). Numerous inflammatory mediators, e.g., interleukins, tumor necrosis factor, histamine, leukotrienes, and prostaglandins, have been found in the MEF of children with AOM (11, 14, 21, 77, 86, 139). Recent studies suggest that virus-induced increased production of various inflammatory mediators in the middle ear is the most plausible explanation for the adverse effect of viruses on the resolution of otitis media.

Histamine is a potent mediator of inflammation that causes vasodilatation and increased vascular permeability (52). In a study of 248 children with AOM, the concentrations of histamine in the MEF were higher in children with evidence of concurrent viral infection than in those without, both in bacterium-positive and bacterium-negative cases (32). Bacteria and viruses together appeared to have an additive effect on histamine content in the MEF, and antibiotic treatment for 3 to 5 days did not reduce the histamine concentrations.

IL-8 is a potent neutrophil-chemotactic cytokine, and leukotriene B₄ (LTB₄) is an inflammatory product of neutrophils related to the degree of inflammation (78, 93). The concentrations of IL-8 and LTB₄ were determined in MEF specimens from 106 children with AOM (33). The highest concentrations of both mediators were measured in MEF specimens that contained both bacteria and virus (Fig. 4). Five of the children had bilateral AOM with identical presence or absence of bacteria in the MEF. The concentrations of IL-8 and LTB₄ were consistently higher in the ear that contained the virus than in the opposite ear without virus. High concentrations of LTB₄ at the initiation of antibiotic treatment were associated with an increased rate of treatment failure. Antibiotic treatment had no effect on the concentrations of these mediators.

In a study quantifying the concentrations of macrophage inflammatory protein-1 α and monocyte chemotactic protein 1, which are both considered histamine-releasing chemokines,

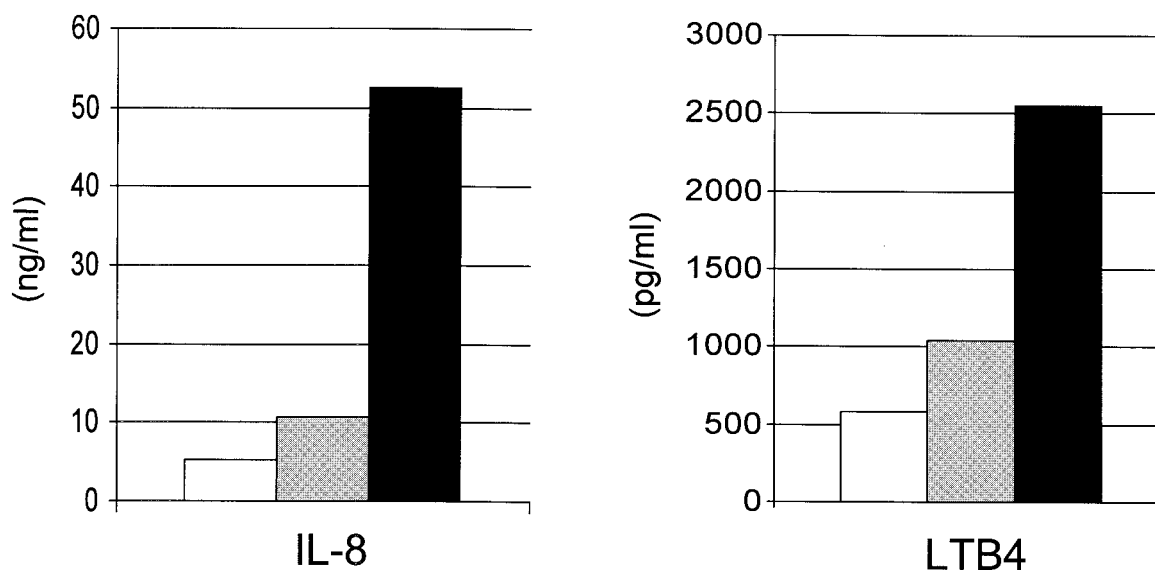


FIG. 4. Concentrations of IL-8 and LTB4 in MEF specimens of children with AOM according to the presence of viruses or bacteria in MEF. Open bars, viruses only; grey bars, bacteria only; black bars, both viruses and bacteria in the MEF. Data derived from reference 33.

higher levels of both mediators were found in MEF specimens with both bacteria and viruses than in specimens with bacteria alone (103). A similar trend has been observed with tryptase concentrations in the MEF, but because of small sample size the differences were not statistically significant (55).

Delayed clearance of bacteria. Studies with both humans and experimental animals have provided convincing evidence for virus-induced dysfunction of the Eustachian tube (38, 59, 112, 138). The impaired drainage to the nasopharynx of middle ear secretions produced during AOM could be thought to mechanically delay the clearance of bacteria from the middle ear cavity. Another potential mechanism for delayed bacterial clearance is the dysfunction of polymorphonuclear leukocytes that has been documented to occur during influenza A virus infections in both chinchillas and humans (1, 83, 115). This neutrophil dysfunction is associated with decreased chemiluminescence, chemotaxis, and bactericidal activity, which could affect the killing of bacteria in the middle ear.

Decreased penetration of antibiotics. It has been hypothesized that viral infection of the middle ear mucosa causes local inflammation that interferes with the penetration of antibiotics into the middle ear, resulting in low concentrations of antibiotics in the MEF. In an experimental animal model, reduced penetration of antibiotics into the middle ear has been shown in animals with pure viral and mixed viral-bacterial infections, compared with animals with bacterial infections alone (76). In humans, the pharmacokinetics of amoxicillin was studied in 30 children with AOM (29). The mean concentrations of amoxicillin were highest in children with bacterial infection, lower in children with combined viral and bacterial infection, and lowest in those with viral infection only. However, the small sample size in the study prevented any firm conclusions, and further studies to confirm the findings are needed.

PREVENTION OF AOM

Based on ample evidence that viral infection plays a decisive role in the initiation of events that finally lead to development of AOM, prevention or effective attenuation of the viral infection could be expected to reduce the incidence of AOM as a complication of the viral illness.

Viral Vaccines

Perhaps the most convincing evidence for the role of respiratory viruses in the development of AOM has been derived from direct interventional studies investigating the efficacy of viral vaccines in the prevention of AOM. In the first of these trials, including 374 children attending day care, half of the children received the trivalent inactivated influenza vaccine before the expected influenza epidemic (70). During the influenza outbreak, the children were examined during each episode of respiratory infection and nasopharyngeal specimens were obtained for viral detection. In children who received the vaccine, the incidence of AOM associated with influenza A virus was decreased by 83%, which resulted in a 36% reduction in the overall incidence of AOM regardless of the viral etiology. The efficacy of the vaccination approach was later confirmed in another study of 186 children in day care (39). Children who had received the influenza vaccine had 32% fewer episodes of AOM during the influenza outbreak. The efficacy of the newly developed live, attenuated intranasal influenza vaccine was recently studied among 1,602 children between 15 and 71 months old (13). The efficacy of the vaccine against influenza was 93%, and the incidence of febrile AOM was reduced by 30% in the children who received the vaccine. Another study using the intranasal virosomal influenza vaccine demonstrated an overall 44% efficacy of vaccination in preventing AOM during a 6-month follow-up period (88). A recent study assessing the efficacy of inactivated influenza vac-

cine failed to show a reduction in the proportion of children with at least one episode of AOM during an entire respiratory season (A. Hoberman, D. P. Greenberg, J. L. Paradise, H. E. Rockette, J. Lave, D. Kearney, D. K. Colborn, M. Kurs-Lasky, M. A. Haralam, C. Byers, L. Zoffel, I. Fabian, B. Bernard, and J. Kerr, *Abstr. Pediatr. Acad. Soc. Annu. Meet.*, abstr. 906, 2002). The design of this study and the subject population differed from those of the previous studies: the efficacy of vaccination in the prevention of influenza-associated AOM was not reported; the subjects were younger than those in other studies (75% of them were younger than 18 months); and they were not all day care attendees, who generally are at higher risk of exposure to infection. More importantly, the influenza activity was low during the study period, and influenza vaccine was not proven effective during the second season of the study.

Unfortunately, influenza vaccine is currently the only vaccine available for the control of respiratory viruses. It is very likely that also other vaccines against major respiratory viruses, especially RSV, would be effective in preventing the development of AOM as a complication. Several types of vaccines against RSV and parainfluenza viruses are currently being developed and some of them are already in early clinical trials (41, 85, 117).

Viral Immune Globulins

Epidemiologic studies have suggested that high levels of transplacentally acquired neutralizing antibodies protect infants from severe RSV infections (60), and passive administration of antibodies has been proposed as one way to prevent AOM. The incidence of AOM was retrospectively analyzed in children (median age, 6 months) who had participated in a clinical trial of RSV-enriched immune globulin (116). The occurrence of AOM was significantly lower in the high-dose group compared with the controls, but the potential confounding effects of tympanostomy tubes, antibiotic prophylaxis, and the presence of antibacterial antibodies in the immune globulin preparation were not ruled out. In addition, it is obvious that this approach that requires monthly intravenous injections is not a feasible way of AOM prevention in everyday clinical practice.

Palivizumab, a recombinant humanized monoclonal antibody directed against the F glycoprotein of RSV, has been shown to reduce RSV-related hospitalizations in premature children (74a). However, palivizumab does not prevent RSV infection and it has not been shown to reduce the incidence of AOM. In a proof-of-concept study, intranasally administered polyclonal immune globulin containing high titers of RSV immunoglobulin A and immunoglobulin G antibodies reduced episodes of rhinitis in children, but the small sample size prevented any conclusions from being made about prevention of AOM as a complication (68).

Antiviral Agents

In principle, antiviral agents could be used in both prevention and treatment of the viral infection. Sufficient attenuation of the viral illness with antiviral drugs could be expected to suppress the inflammatory response, which, in turn, could lead

to decreased incidence of complications such as AOM. At present, specific antiviral treatments for respiratory viruses are commercially available only for influenza viruses (65, 95). One study has evaluated the efficacy of oseltamivir, a new influenza neuraminidase inhibitor, in the prevention of AOM in children (137). The study included 695 children 1 to 12 years old who had acute respiratory symptoms of less than 48 h of duration during an influenza epidemic. Influenza virus infection could be confirmed in 65% of the children. In influenza-infected children, oseltamivir treatment provided a 44% reduction in the development of AOM compared with that observed for children receiving placebo. The results of this study indicate that early treatment of viral infection with a specific antiviral drug can prevent the development of AOM, and similar efficacy might be seen also with antiviral drugs against other common viruses that predispose individuals to AOM. However, even with the availability of effective antivirals against all major viruses, two practical problems remain. First, the clinical manifestations of different viral infections are usually indistinguishable, especially in children, and the optimal use of virus-specific antiviral agents would require positive identification of the specific virus causing the infection. Second, the initiation of antiviral treatment should occur as soon as possible after the onset of respiratory symptoms and at the latest within 48 h. The need to see a doctor already in the early course of illness might cause problems in many health care systems.

CONCLUSIONS

There is growing evidence that the etiopathogenesis of AOM is far more complicated than is currently understood, consisting of a network of factors affecting each other. It is also probable that many essential factors involved in the disease process still remain to be identified. Further research into the host inflammatory response and mechanisms of viral-bacterial interaction is needed to elucidate pathways that could serve as targets for intervention to improve the treatment of AOM or to prevent the disease. This need is underscored by the increasing antimicrobial resistance of bacteria that may increase the pressure against routine use of antibiotics in all cases of AOM. More information is also required about the effect of specific respiratory viruses on the resolution of otitis media and the potential role of antiviral agents and adjunctive treatments in improving the outcome. The availability of vaccines against the major respiratory viruses predisposing to AOM would likely be the most effective way to prevent AOM.

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